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The Rate of Photorespiration during Photosynthesis and the Relationship of the Substrate of Light Respiration to the Products of Photosynthesis in Sunflower Leaves[1]

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ABSTRACT

Single attached leaves of sunflower (*Helianthus annus* L. "Mennonite") were supplied $^{14}CO_2$ of constant specific radioactivity in gas mixtures containing various CO_2 and O_2 concentrations. The $^{14}CO_2$ and CO_2 fluxes were measured concurrently in an open system using an ionization chamber and infrared gas analyzer.

The rate of photorespiration (5.7 ± 0.3 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$) during photosynthesis in 21% O_2 at 25 C and 3,500 footcandles was over three times the rate of dark respiration and was independent of CO_2 concentrations from 0 to 300 μl/l. The steady rate of CO_2 evolution into CO_2-free air was about 30% lower. Low oxygen (1%) inhibited both $^{14}CO_2$ and CO_2 evolution, both during photosynthesis and in CO_2-free air in the light.

At 300 μl/l CO_2 apparent photosynthesis was inhibited 41% by 21% O_2. Two-thirds of the inhibition was due to the inhibition of true photosynthesis by oxygen and one-third due to the stimulation of photorespiration. At 50 μl/l CO_2, where the percentage inhibition of apparent photosynthesis by 21% oxygen was 92%, photorespiration accounted for two-thirds of the total inhibition.

The rate of $^{14}CO_2$ uptake by the leaf decreased about 30 seconds after the introduction of $^{14}CO_2$, indicating that $^{14}CO_2$ was rapidly evolved from the leaf. The rate of $^{14}CO_2$ evolution increased rapidly with time, the kinetics depending on the CO_2 concentration. The high specific radioactivity of the $^{14}CO_2$ evolved during photosynthesis or in the early period of flushing in CO_2-free air showed that the substrate for light respiration was an early product of photosynthesis. From the measurement of $^{14}CO_2$ and CO_2 evolution into CO_2-free air over a longer time period it was apparent that at least three compounds, each of decreased ^{14}C content, could supply the substrate for light respiration.

Based on a consideration of the specific radioactivity of $^{14}CO_2$ evolved under a variety of conditions, it is suggested that total CO_2 evolution in the light or photorespiration is composed of two processes, dark respiration and light respiration. Light respiration is a process that only occurs in the light, persists for some time on darkening, and metabolizes substrates that are quite different from those of dark respiration.

There is now much evidence to show that the rate of CO_2 evolution from green leaves in the light exceeds that of dark respiration and that the substrate and mechanism of CO_2 evolution in the light are different from those of CO_2 evolution in the dark (24). All methods to date (23, 24, 38), however, have not measured the rate of photorespiration under conditions of steady state photosynthesis nor have they (18, 38) allowed a continuous measurement of the specific radioactivity of the $^{14}CO_2$ evolved from a leaf during or after a period of photosynthesis in $^{14}CO_2$. We have recently described a system (26) which continuously measures the CO_2 or $^{14}CO_2$ fluxes from leaves. In this paper we present results on the rates of photorespiration during steady state photosynthesis and on the relationship of the $^{14}CO_2$ evolved to the products of $^{14}CO_2$ fixation. Brief accounts of this work have previously been presented (27, 28).

MATERIALS AND METHODS

The materials and methods have been fully described in a preceding paper (26). Sunflower leaves (*Helianthus annuus* L. "Mennonite"), grown as previously described (26), were used for all experiments.

RESULTS

From the CO_2 and $^{14}CO_2$ uptake and the average specific radioactivity of $^{14}CO_2$ in the leaf chamber the rates of true photosynthesis, apparent photosynthesis and photorespiration of sunflower leaves were determined (Fig. 1). The estimated rate of true CO_2 uptake in 21% O_2 was consistently about 5.7 ± 0.3 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$ higher than the rate of apparent CO_2 uptake (Fig. 1). The rate of CO_2 evolution, represented by this difference, was therefore independent of the CO_2 concentration and the rate of photosynthesis. A similar estimate of the rate of CO_2 evolution was obtained when the rate of apparent photosynthesis in 21% O_2 was extrapolated to zero CO_2 concentration.

The rates of apparent CO_2 uptake in 1% O_2 were considerably higher than the rates in 21% O_2 at comparable CO_2 concentrations. The estimated rates of true CO_2 uptake in 1% O_2 were not significantly different to the apparent rates and there was no measurable CO_2 evolution (Fig. 1). It is clear from these results that an increase in the oxygen concentration had two effects on the CO_2 exchange of a sunflower leaf; the rate of true CO_2 uptake was inhibited, and the rate of CO_2 evolution was stimulated. The net effect was a considerable decrease in the rate of apparent CO_2 uptake.

As shown in a previous publication (26), $^{14}CO_2$ uptake was maximum 30 sec after the introduction of $^{14}CO_2$ to the leaf

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chamber and then decreased as time in $^{14}CO_2$ was extended. This decrease in $^{14}CO_2$ uptake was ascribed to $^{14}CO_2$ evolution, and the amount of $^{14}CO_2$ evolution at any time could be calculated (26). Since the CO_2 concentration in the leaf chamber was constant with time and steady rates of apparent CO_2 uptake were measured, it was assumed that the rate of CO_2 evolution was constant also over the $^{14}CO_2$ uptake period. Thus, from the rates of $^{14}CO_2$ and CO_2 evolution the specific radioactivity of the $^{14}CO_2$ evolved could be calculated. The specific radioactivity of the $^{14}CO_2$ evolved from the leaf was expressed as a percentage of the average specific radioactivity of $^{14}CO_2$ in the chamber, and this value was termed the relative specific radioactivity.

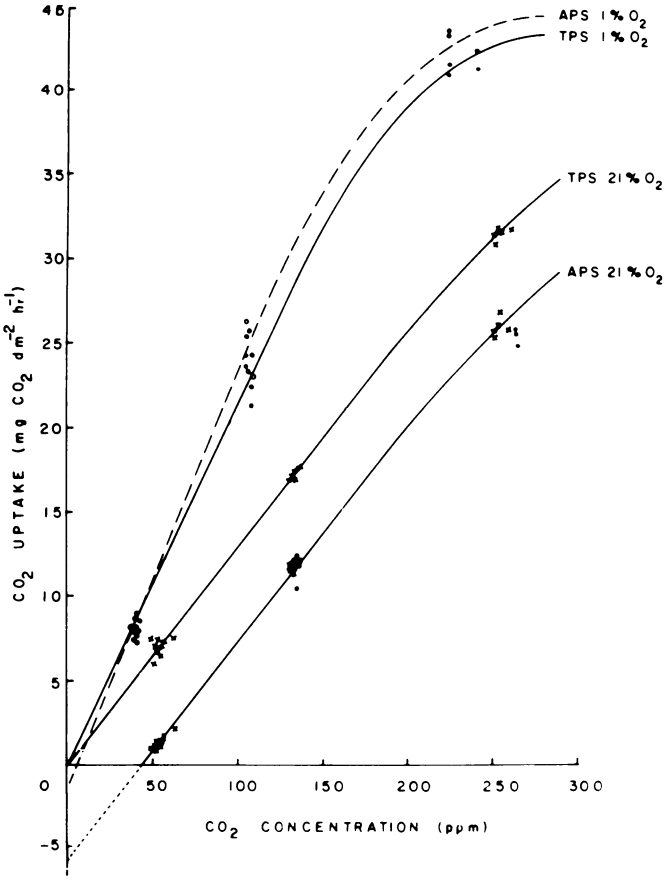

FIG. 1. Effects of CO_2 (average in the leaf chamber) and O_2 concentration on the rate of true photosynthesis, apparent photosynthesis and photorespiration of sunflower leaves.

The effect of CO_2 concentration on the relative specific radioactivity of $^{14}CO_2$ evolved from the leaf is clearly shown in Figure 2. $^{14}CO_2$ evolution from the leaf was observed at all CO_2 concentrations within 1 min after $^{14}CO_2$ was supplied to the leaf. In 290 μl/l CO_2 the specific radioactivity of the evolved $^{14}CO_2$ rapidly increased, and within 7 min it had approached the specific radioactivity of the supplied $^{14}CO_2$. Thereafter, it remained constant. In 150 μl/l CO_2, the relative specific radioactivity increased rapidly to a value of 70% and then slowly increased during the remainder of the experimental period. In 53 μl/l CO_2, the relative specific radioactivity of the evolved $^{14}CO_2$ increased rather slowly and appeared to equilibrate partially at a value of 60%.

At the end of the $^{14}CO_2$ uptake period (60 min), the leaf chamber was flushed with CO_2-free air, and the rates of $^{14}CO_2$ and CO_2 evolution were determined directly. The specific radioactivity of the $^{14}CO_2$ evolved from the leaf was calculated and related to the specific radioactivity of the $^{14}CO_2$ previously supplied to the leaf chamber. In CO_2-free air the relative specific radioactivity decreased rapidly, but the value determined in the first minute of flushing agreed remarkably well with the value calculated from the $^{14}CO_2$ uptake data (Fig. 2) just prior to flushing. The close agreement between the measured and calculated values indicated that the calculations (26) provided a reliable estimate of the relative specific radioactivity of the $^{14}CO_2$ evolved during photosynthesis.

More extensive results of flushing the leaf after 60 min photosynthesis in $^{14}CO_2$ with CO_2-free air or with normal air in the light and dark are presented in Figures 3, 4 and 5. The rates of CO_2 evolution in CO_2-free air in the light were similar

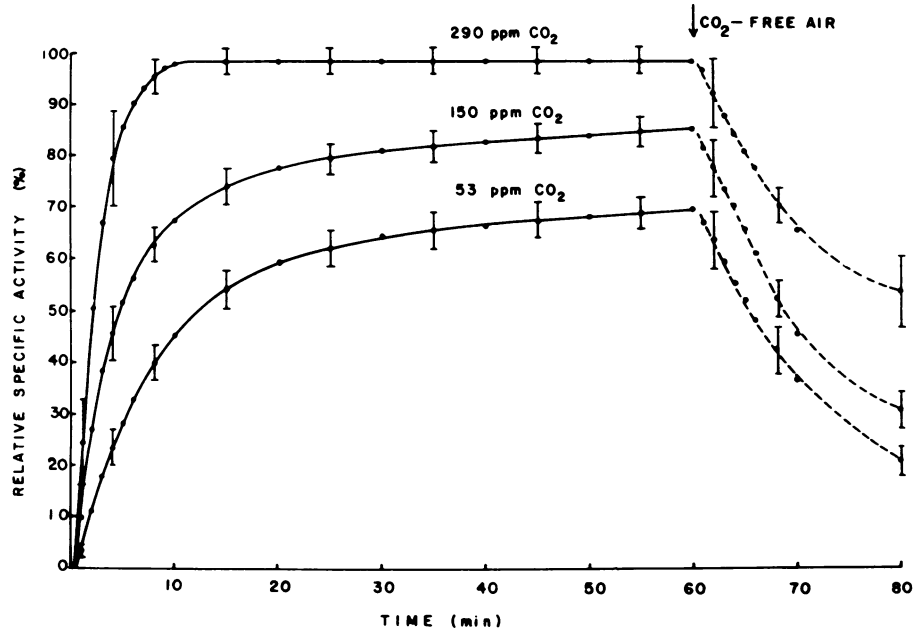

FIG. 2. Effect of CO_2 concentration on the relative specific radioactivity of $^{14}CO_2$ evolved by sunflower leaves during photosynthesis in $^{14}CO_2$. (Results from five experiments at each CO_2 concentration; vertical lines represent 95% confidence intervals).

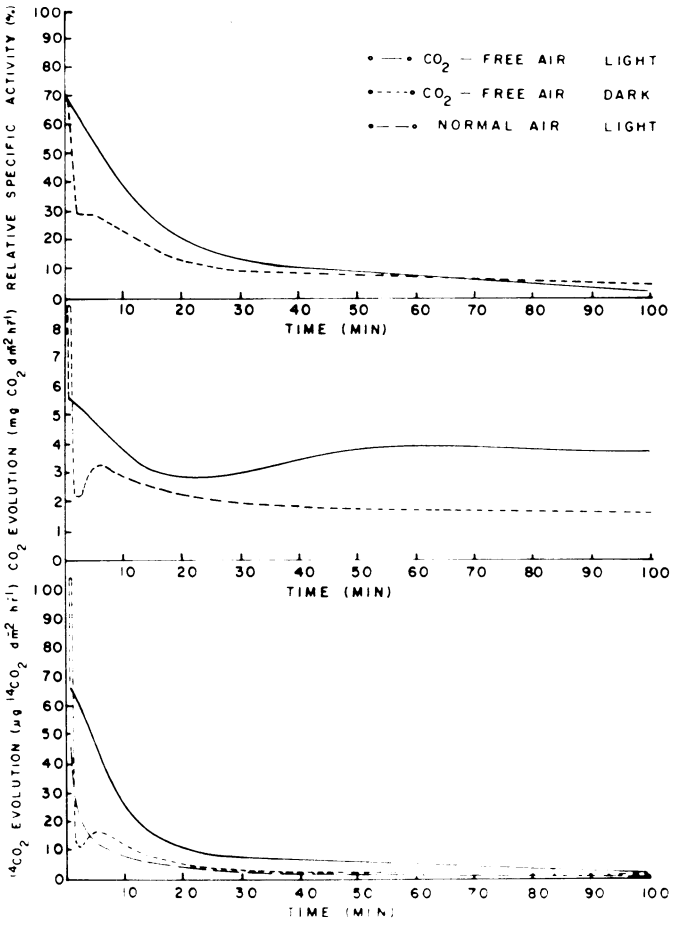

FIG. 3. $^{14}CO_2$ and CO_2 evolution by illuminated or darkened sunflower leaves following 60 min photosynthesis in $^{14}CO_2$ and 53 μl/l CO_2.

in the three experiments and in the first few minutes of flushing were between 5 and 6 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$. These rates (Figs. 3–5) agreed very well with the rates estimated using $^{14}CO_2$ during photosynthesis (Fig. 1) and support the conclusion that the CO_2 concentration and the rate of photosynthesis have little effect on the rate of CO_2 evolution. The rate of CO_2 evolution did not remain constant, however, but decreased, with some fluctuation, to a steady rate of 4 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$. The slow fluctuations in rate during the first 60 min of flushing were not always observed and, at present, cannot be explained. The transpiration rate was constant over this period indicating that changes in stomatal aperture were not involved.

When the leaf was flushed with CO_2-free air in the dark, the rates of CO_2 evolution were similar in the three experiments and two bursts of CO_2 were apparent. The first burst occurred in the first minute of darkness and the maximum rate of CO_2 evolution was 8 to 9 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$. A second, smaller burst followed a few minutes later, and then the rate slowly decreased to about 1.7 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$.

The patterns of $^{14}CO_2$ evolution when illuminated or darkened leaves were flushed with CO_2-free air were similar to the patterns of CO_2 evolution, but there were several major differences. The rate of $^{14}CO_2$ evolution in the light was initially high and steadily decreased during the first 15 min of flushing. When the CO_2 concentration was 53 μl/l during the $^{14}CO_2$ uptake period (Fig. 3), the rate of $^{14}CO_2$ evolution, after the initial rapid decrease, slowly decreased to a very low rate, but when the previous CO_2 concentration was 335 μl/l (Fig. 5) a very considerable increase in the rate of $^{14}CO_2$ evolution was observed after the first 15 min. The maximum rate occurred after about 60 min in CO_2-free air and then slowly decreased. A comparable small increase in the rate of $^{14}CO_2$ evolution was observed when the previous CO_2 concentration was 150 μl/l (Fig. 4). A comparison of $^{14}CO_2$ evolution in the light and in darkness shows that the rates of $^{14}CO_2$ evolution in the light were very much higher than the rates in darkness, except for the dark outbrust in the first minute. Similar results have been presented by Goldsworthy (18) and Zelitch (38, 39).

When an illuminated leaf was flushed with a gas mixture containing CO_2, the rate of $^{14}CO_2$ evolution decreased more rapidly than in CO_2-free air and soon reached a low rate.

The previous CO_2 concentration had a considerable effect on the relative specific radioactivity of $^{14}CO_2$ evolved into CO_2-free air in light and in darkness. When the previous CO_2 concentrations were 53 μl/l, 150 μl/l, and 335 μl/l (Figs. 3–5) the relative specific radioactivities in both light and darkness were initially about 68%, 82%, and 97%, respectively, values very close to the values calculated during the $^{14}CO_2$ uptake period (Fig. 2). With continued flushing in the light, the relative specific radioactivity decreased in all experiments. When the previous CO_2 concentration was 53 μl/l, the specific radioactivity decreased steadily. When the previous CO_2 concentration was 150 μl/l, the relative specific radioactivity steadily declined to about 30% where it remained for about 20 min before declining further. When the previous CO_2 concentration was 335 μl/l, the relative specific radioactivity decreased to about 60% where it remained for a considerable period before

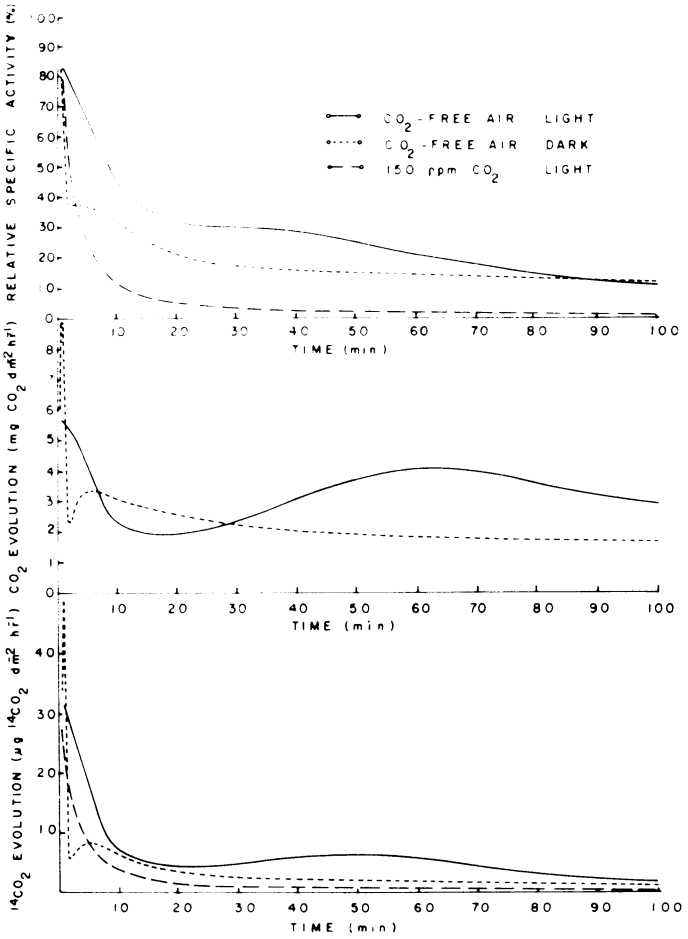

FIG. 4. $^{14}CO_2$ and CO_2 evolution by illuminated or darkened sunflower leaves following 60 min photosynthesis in $^{14}CO_2$ and 150 μl/l CO_2.

decreasing further. In the dark, immediately following the first outburst, the relative specific radioactivity decreased very rapidly in all experiments.

If the relative specific radioactivities in light and in darkness are compared, the following observations may be made. Initially, at the time of the first outburst, the relative specific radioactivities were the same in light and in darkness. However, following this outburst there was a considerable period when the relative specific radioactivity of $^{14}CO_2$ evolved in the light was considerably higher than the relative specific radioactivity in darkness, particularly in experiments where the previous CO_2 concentration was high (Fig. 5). Eventually the relative specific radioactivity in the light declined to equal that in the dark. This occurred after about 60 min, when the previous CO_2 concentration was 53 μl/l, 90 min when it was 150 μl/l, and several hours when it was 335 μl/l.

In the foregoing results, it appeared that the relative specific radioactivity of the $^{14}CO_2$ evolved into CO_2-free air following a period of photosynthesis in $^{14}CO_2$ depended upon the CO_2 concentration and thus probably upon the amount of CO_2 fixed. To further investigate this aspect, $^{14}CO_2$ was supplied, the leaf was flushed with CO_2-free air in the light, and the relative specific radioactivity of the evolved $^{14}CO_2$ was determined (Fig. 6).

The relative specific radioactivities measured in the first minute of flushing (Fig. 6) agreed closely with expected values determined from the calculated data shown in Figure 2 and supported the conclusion that the substrate(s) for CO_2 evolution during photosynthesis in 53 to 56 μl/l or 298 to 335 μl/l CO_2 were highly labeled after short periods in $^{14}CO_2$.

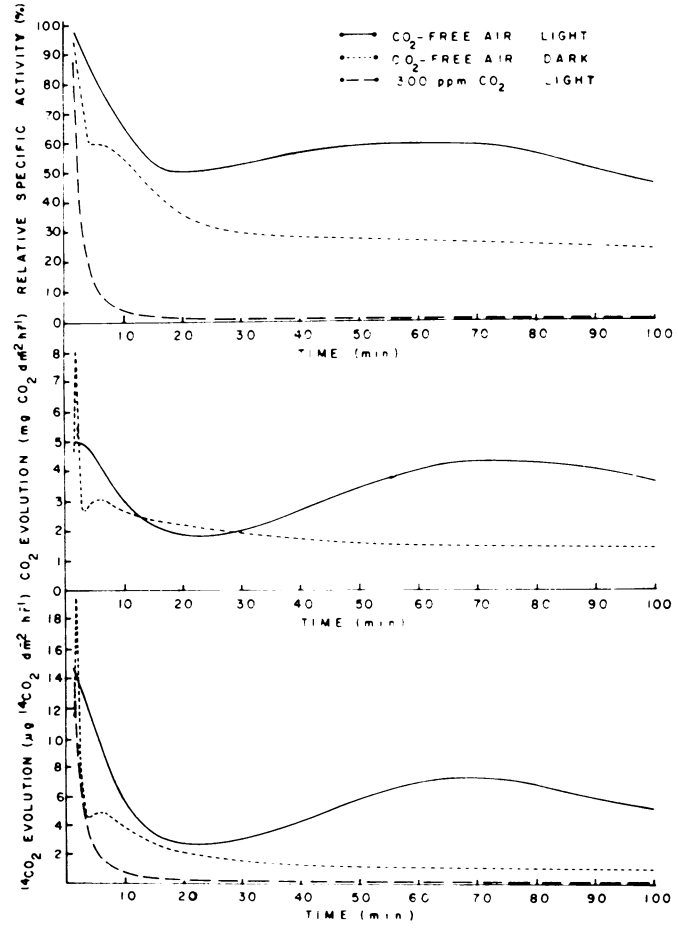

FIG. 5. $^{14}CO_2$ and CO_2 evolution by illuminated or darkened sunflower leaves following 60 min photosynthesis in $^{14}CO_2$ and 335 μl/l CO_2.

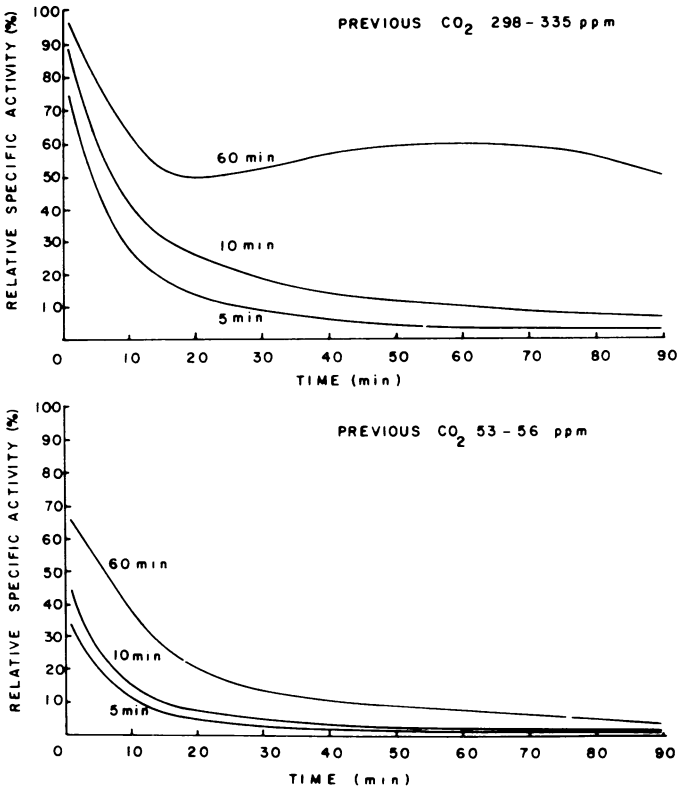

FIG. 6. The relative specific radioactivity of $^{14}CO_2$ evolved into CO_2-free air in the light following various periods of photosynthesis in $^{14}CO_2$. The CO_2 concentration and time of photosynthesis are shown on the graph.

When the previous CO_2 concentration was 53 to 56 μl/l, the relative specific radioactivity steadily decreased to a low value (Fig. 6). Similar results were obtained when the period of $^{14}CO_2$ uptake in 298 to 335 μl/l was short (5 or 10 min), but when the uptake period was increased to 60 min the relative specific radioactivity decreased steadily to about 60% where it remained for a considerable period of time.

These results therefore show that the relative specific radioactivity of the evolved $^{14}CO_2$ was proportional to the total CO_2 fixation and that continued evolution of high specific radioactivity $^{14}CO_2$ occurred only when the CO_2 concentration during the $^{14}CO_2$ uptake period was high, and only after relatively long periods in $^{14}CO_2$, *i.e.*, only when total $^{14}CO_2$ fixation was large.

Similar experiments were carried out to determine the effect of total CO_2 fixation on the relative specific radioactivity of the $^{14}CO_2$ evolved into CO_2-free air in darkness (25). As the total fixation of $^{14}CO_2$ increased, the relative specific radioactivity of the $^{14}CO_2$ evolved during the first dark outburst increased. The labeling patterns were similar to those shown in Figure 2 and suggested that the $^{14}CO_2$ evolved during the dark outburst was of similar origin to that evolved during photosynthesis. The relative specific radioactivity of the $^{14}CO_2$ evolved in steady dark respiration was low in all experiments and extremely long periods in $^{14}CO_2$ would be required before the specific radioactivity of $^{14}CO_2$ evolved in the dark completely equilibrated with the $^{14}CO_2$ supplied.

To determine further the effect of various amounts of CO_2 fixation on the specific radioactivity of the $^{14}CO_2$ subsequently evolved into CO_2-free air in the light, CO_2 fixation was varied

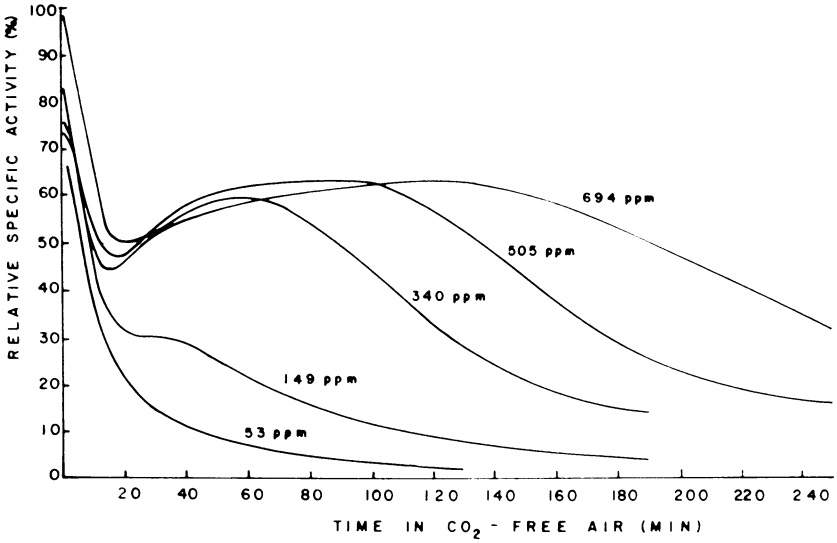

FIG. 7. Effects of the previous CO_2 concentration on the relative specific radioactivity of $^{14}CO_2$ evolved into CO_2-free air in the light. O_2 concentration during photosynthesis was 21% and time of photosynthesis in $^{14}CO_2$ was 60 min.

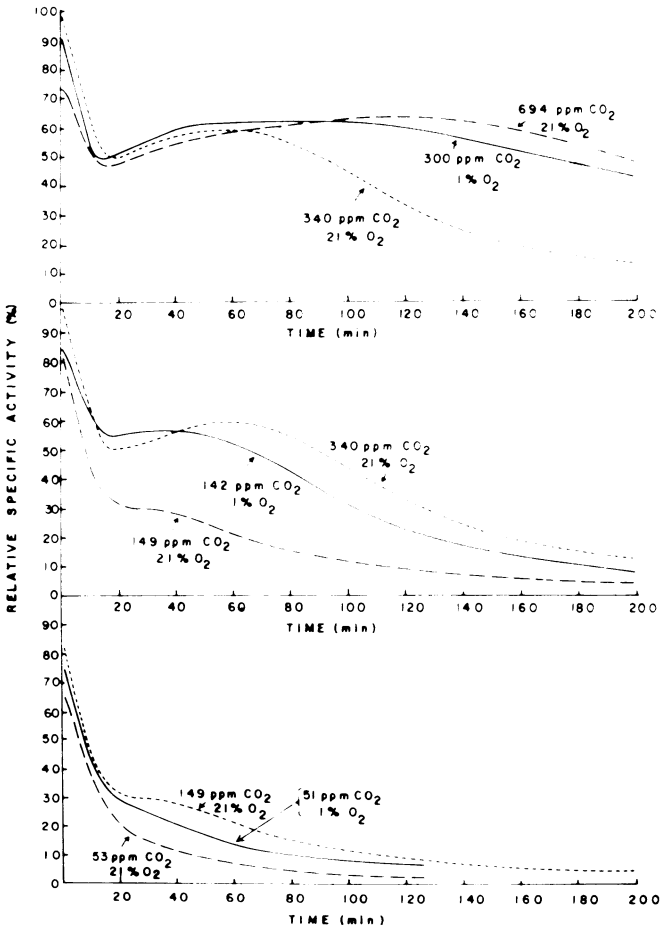

FIG. 8. Effects of the CO_2 and O_2 concentration (indicated on graph) during fixation on the relative specific radioactivity of $^{14}CO_2$ evolved into CO_2-free air in the light. All $^{14}CO_2$ fixation periods were 60 min.

by changing the CO_2 or O_2 concentrations (Figs. 7 and 8). At 53 and 149 μl/l CO_2 the changes in specific radioactivity (Fig. 7) were similar to those already described (Fig. 3 and 4) and indicated a rapid change from the utilization of a radioactive substrate during the photosynthetic period to a nonradioactive substrate in CO_2-free air. When the CO_2 concentration during the $^{14}CO_2$ uptake period was 340 μl/l or higher, however, a second substrate could be readily distinguished. The amount of this second substrate was increased after $^{14}CO_2$ fixation in 505 or 694 μl/l CO_2, but there was little increase in its specific radioactivity (Fig. 7).

When the fixation was varied by decreasing the oxygen concentration to 1%, two radioactive and a nonradioactive substrate were again available for CO_2 evolution in CO_2-free air (Fig. 8) in spite of the fact that all $^{14}CO_2$ fixation occurred in 1% O_2 where there was little, if any, $^{14}CO_2$ evolution or CO_2 evolution (Fig. 1). In all three CO_2 concentrations a decrease in the O_2 concentration to 1% during fixation increased the amount of the second radioactive substrate (Fig. 8). One percent O_2 also significantly increased the specific radioactivity of this substrate in the 51 and 142 μl/l CO_2 experiments, but not in the 300 μl/l experiment. In general, at each CO_2 concentration a reduction of the O_2 concentration to 1% had an effect similar to that observed from increasing the CO_2 concentration at 21% O_2 (Fig. 8).

The amount and relative specific radioactivity of the second substrate utilized in CO_2-free air therefore seems to depend on the total carbon fixed during the $^{14}CO_2$ uptake period. When rates of apparent CO_2 uptake were below 10 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$, little of the second substrate was observed. Increasing apparent CO_2 uptake from 10 to 40 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$ resulted in an increased amount of this second radioactive substrate. For example, when the previous rates of CO_2 fixation were 10, 30, and 40 mg CO_2, this second substrate supported high rates of $^{14}CO_2$ evolution in CO_2-free air for about 20, 60, and 140 min respectively, before it was gradually replaced by the third unlabeled substrate. The specific radioactivity of the second substrate increased with increasing fixation up to about 25 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$. Further increases in fixation had little effect.

DISCUSSION

By using the open system described previously (26), reliable measurements of the CO_2 and $^{14}CO_2$ fluxes from leaves in the light were obtained 30 sec after the $^{14}CO_2$ was introduced into the leaf chamber. Since the gas composition and other con-

ditions were constant, the leaf was always under steady rate (and presumably steady state) conditions of photosynthesis.

The observed changes in $^{14}CO_2$ or CO_2 fluxes could not be ascribed to changes in stomatal aperture as the transpiration rate of leaves in the light was not affected by the O_2 concentration and was only slightly affected by the CO_2 concentration. Stomata were open in all treatments as indicated by the low laminar and stomatal resistances ($r_a + r_s$) CO_2, 0.68 sec·cm^{-1} in CO_2-free air and 0.78 sec·cm^{-1} in 340 μl/l CO_2. Gauhl and Bjorkman (16) and D'Aoust (8) have also shown that the transpiration rate of leaves was not affected by the O_2 concentration.

The rate of photorespiration of sunflower leaves was not affected by the rate of photosynthesis or CO_2 concentration over a range of 0 to 300 μl/l CO_2 as the same rate of 5.7 $\pm$ 0.3 mg CO_2·dm^{-2}·hr^{-1} was measured by the $^{14}CO_2/CO_2$ technique (Fig. 1) by evolution into CO_2-free air (Fig. 3, 4, and 5) or by extrapolation of the rate of apparent photosynthesis to zero CO_2 (Fig. 1). It is clear that maximum rates of CO_2 evolution into CO_2-free air are only obtained in the first 5 min of flushing, and thereafter the rate decreases by about 30% (Figs. 3–5). It is also clear that maximum rates of CO_2 evolution are measured by the $^{14}CO_2/CO_2$ technique only if the measurements are performed within the first minute after $^{14}CO_2$ is introduced into the leaf chamber (12, 23). If the measurements are not performed until 2 min after $^{14}CO_2$ is supplied to the leaf, the amount of CO_2 evolution will be underestimated 10% at 53 μl/l and will be underestimated 50% at 300 μl/l (Fig. 2). Bidwell *et al.* (5) have presented data, obtained with a closed system, showing that the rate of photorespiration in bean leaves at 40 to 80 μl/l CO_2 was four times the rate of photorespiration at 300 to 400 μl/l CO_2. Whereas bean leaves may well be different from sunflowers, the results they obtained are precisely those expected if the initial $^{14}CO_2$ uptake was not determined until 2 to 3 min had elapsed after $^{14}CO_2$ was first introduced to the leaf (36).

It is well established for many plants (21, 24) that the rate of apparent photosynthesis in 21% O_2 is considerably less than the rate of apparent photosynthesis in 1% O_2. Tregunna *et al.* (35) and Forrester *et al.* (15) concluded that the inhibition of apparent photosynthesis by O_2 was due to two different effects, a stimulation of photorespiration and an inhibition of true photosynthesis. In the present study, it was possible, for the first time, to measure directly the separate effects of oxygen on true photosynthesis and concurrent photorespiration (Fig. 1). Photorespiration was not affected by CO_2 concentration but true photosynthesis was, so that as the CO_2 concentration changed the relative importance of O_2 on these processes also altered. Thus, at 50 μl/l CO_2, the inhibition of true photosynthesis accounted for 33% of the total inhibition of apparent photosynthesis and photorespiration for the remaining 66%. At 300 μl/l CO_2 68% of the total inhibition could be ascribed to an inhibition of true photosynthesis and only 32% to photorespiration. Curtis *et al.* (7) calculated that the total inhibition of apparent photosynthesis by oxygen could be about equally ascribed to an inhibition of true photosynthesis and a stimulation of photorespiration. Because of these dual effects of oxygen, it does not appear valid to assume that the difference between the rate of apparent photosynthesis in 1% and 21% O_2 is entirely due to photorespiration (14, 21), as a direct effect of O_2 on photosynthesis (17) could also be involved.

The specific radioactivity of the $^{14}CO_2$ evolved during photosynthesis or in the first minute of flushing with CO_2-free air showed quite clearly that during these times a very large proportion of the substrate for photorespiration was an immediate product of photosynthesis (Fig. 2). After longer flushing in CO_2-free air in the light, however, a more complex

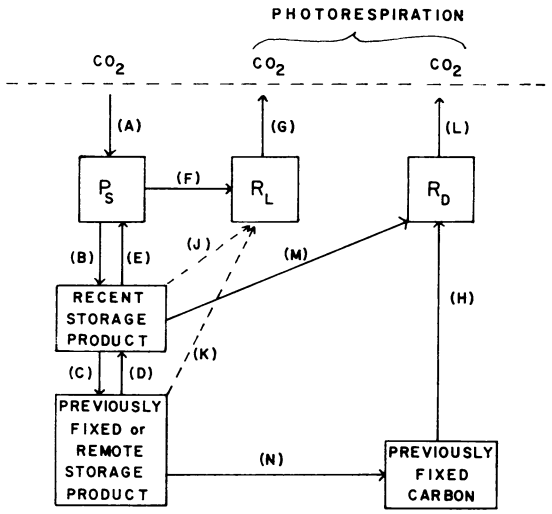

FIG. 9. Diagrammatic representation showing carbon flow (arrows) and relationships between the carbon compounds of photosynthesis (P_S), light respiration (R_L), and dark respiration (R_D). For further details see text.

picture was presented, as CO_2 evolution did not cease and $^{14}CO_2$ evolution varied markedly with the amount of $^{14}CO_2$ fixation that occurred in the previous photosynthetic period. For further discussion of the specific radioactivity changes a diagrammatic scheme showing compartments and direction of carbon flow is presented in Figure 9. The compartments are defined more in conceptual terms rather than in physical or chemical terms. Photosynthesis (P_S), physically located in the chloroplast (2, 20) includes the site of CO_2 fixation and the chemical intermediates of photosynthesis (2). Light respiration (R_L) includes the site of CO_2 release in the light and its immediate substrate. It has been suggested that CO_2 release in the light involves the chloroplast (5) or the peroxisomes and mitochondria (34), and that the substrate is glyoxylate (37) or glycine (34). Dark respiration (R_D) includes the compounds of the dark respiratory pathways (3). Photorespiration, or the accelerated CO_2 evolution that is observed in the light, is composed of CO_2 from light respiration (R_L) and dark respiration (R_D). Recent storage products represents soluble storage compounds from photosynthesis. Previously fixed carbon represents soluble precursors of dark respiratory pathways. Previously fixed or remote storage products represent more remote (perhaps insoluble) forms of carbon storage.

For purposes of discussion it is assumed that dark respiration (R_D) continues at the same rate in the light. Some data are available to indicate that dark respiration may be suppressed in the light (24) but there is also evidence, based on the metabolism of ^{14}C-labeled intermediates, that shows that the dark respiration pathways continue to function in the light (19, 29). There does not appear to be a direct effect of light on dark respiration in leaves as the rate is not affected by light when photosynthesis is suppressed by lack of chlorophyll (22) or by inhibitors (10, 11). Thus in the absence of definitive evidence that dark respiration is inhibited it seems reasonable to assume that it continues.

Whereas the rate of dark respiration may not be affected by light, there is a considerable amount of evidence (1, 4, 19, 31) that shows that the substrates for dark respiration do not become labeled or become labeled very slowly with ^{14}C when $^{14}CO_2$ is supplied to the leaf in the light. If a dark period is imposed on the leaf after a period of $^{14}CO_2$ fixation in the light, however, label rapidly enters the compounds of the tricarboxylic acid pathway (1, 4, 19, 31, 33) and $^{14}CO_2$ will be evolved from the dark respiration pathways.

Light respiration (R_L) is a process that only occurs in the light, persists for a short time on darkening, and is physically and chemically distinct from the process of dark respiration. It is assumed that only a single site and a single substrate for CO_2 release is involved, although multiple sites and substrates for CO_2 release cannot be excluded. It is also assumed that there is a single pathway for formation of the substrate, although again there is no evidence to rule out multiple parallel pathways. It is suggested by the results (Fig. 2), that, when the rate of photosynthesis is adequate, the substrate is supplied directly from the immediate products of CO_2 fixation via F (Fig. 9). When photosynthesis is restricted, (*e.g.*, by lack of CO_2) substrate is still generated via (F) but the precursors in (P_S) must now be generated from compounds (*e.g.*, recent storage product) other than CO_2. In these conditions the photosynthetic cycle must assume a respiratory character to generate substrate for decarboxylation from previously fixed compounds.

The CO_2 from both light respiration (R_L) and dark respiration (R_D) will mix intimately in the intercellular spaces and that portion that escapes from the leaf in the light constitutes photorespiration. A certain portion of the CO_2 produced in these two processes will be refixed in photosynthesis and, while it is impossible to know the amount precisely, it is necessary for the interpretation of the $^{14}CO_2$ evolution data (see later) to make some estimate of the amount of refixation. Moss (30) has suggested that the postillumination outburst or first dark surge represents a minimum estimate of total CO_2 production during the light. From our data (Fig. 3 to 5) the dark outburst or total CO_2 production was about 8.5 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$ and the rate of steady CO_2 evolution in the light was 5.6 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$. The difference or refixation was equal to 34% of the total production. Samish and Koller (32) suggest that refixation is 34% and Bravdo (6) suggests that refixation is 21 to 25%. Considering all the estimates it has been assumed for purposes of discussion that refixation is about 35% of the production in 21% O_2.

If, as suggested by Moss (30), total CO_2 production can be estimated from the first dark surge, the rate of CO_2 production in sunflowers in 21% O_2 was 8.5 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$. This was made up of 1.7 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$ from dark respiration and 6.8 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$ from light respiration (8.5–1.7). If the CO_2 from dark respiration was unlabeled and the CO_2 from light respiration was equal in specific radioactivity to the $^{14}CO_2$ supplied, the CO_2 evolved from the leaf would have a relative specific radioactivity of 80% (680 units radioactivity/8.5 units CO_2).

After 15 to 20 min in CO_2-free air, CO_2 evolution in the light decreases to about 3.5 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$ (Figs. 3 to 5). The expected specific radioactivity of evolved $^{14}CO_2$ can also be estimated for this time if it is assumed that this decrease is due to a reduction in the rate of light respiration (possibly because the substrate cannot be generated at the same rate) and that dark respiration is unaffected. Assuming the same amount of refixation (35%) total production will be 4.7 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$. Dark respiration will contribute 1.7 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$ and light respiration 3.0 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$. With the same assumptions as above, the relative specific radioactivity of the evolved CO_2 will be 64% (300 units radioactivity/4.7 units CO_2).

It is suggested that CO_2 evolution during photosynthesis arises via (G) and (L) (Fig. 9). At 53 μl/l CO_2 (Fig. 2) the compounds of P_S are not saturated with ^{14}C, and the relative specific radioactivity after 1 hr of fixation will be less than the calculated 80% and is about 65%. At 150 μl/l CO_2 the compounds of P_S are saturated with ^{14}C, and the relative specific radioactivity is about 80% (Fig. 2). At 300 μl/l CO_2 the $^{14}CO_2$ evolved is close to 100% relative specific radioactivity (Fig. 2). This high specific radioactivity should not be obtained if dark respiration continues (see earlier) and more will be said of it later.

During flushing with CO_2-free air in the light the relative specific radioactivity of the $^{14}CO_2$ initially evolved will be the same as that evolved during photosynthesis (Fig. 2) since the substrates are identical (Fig. 9). The amount of substrate in R_L or P_S, however, is small and is soon exhausted (Figs. 3–5) because it is not replaced via (A). Thus the substrate must soon be diluted by material drawn from recent storage product via (E) (Fig. 9) and the specific radioactivity will fall to the specific radioactivity of these storage products. At the same time the limitation on substrate supply results in a decrease in the rate of R_L. If total net fixation of $^{14}CO_2$ has been small (less than 12 mg $CO_2 \cdot dm^{-2}$) the specific radioactivities of the recent storage products are low and the specific radioactivity of the CO_2 evolved will soon decrease to a low value (Figs. 3, 6, 8). Between 12 and 20 mg $CO_2 \cdot dm^{-2}$ of total fixation, more ^{14}C is fixed into the recent storage products and CO_2 may be evolved into CO_2-free air at a constant specific radioactivity for some time (Fig. 4) before decreasing further. When total fixation reaches 20 mg $CO_2 \cdot dm^{-2}$ the recent storage products are saturated with ^{14}C and further $^{14}CO_2$ fixation results in an increase in the size of this pool and a slow labeling of the remote storage product (Fig. 9). Thus, after such fixations $^{14}CO_2$ will be evolved at a relative specific radioactivity of about 60% (see earlier for reasoning) during a period of flushing in CO_2-free air (Figs. 5, 6, 8) and further fixation will not result in any increase in the specific activity but will prolong the period over which 60% relative specific radioactivity $^{14}CO_2$ is evolved (Figs. 7, 8).

After a prolonged period of flushing in CO_2-free air the pool of recent storage products will be depleted and substrate will then be supplied to R_L from previously fixed material via (D), (E), and (F). This material will not be radioactive (unless there has been an extremely long $^{14}CO_2$ fixation) and thus the relative specific radioactivity of the evolved CO_2 will slowly decrease to zero (Figs. 3, 4, 7, 8).

As indicated earlier, the evolution of $^{14}CO_2$ at 100% relative specific radioactivity in 300 μl/l CO_2 (Fig. 2) is not consistent with the above proposal but is consistent with a complete suppression of dark respiration. This alternative explanation requires, however, a source, other than dark respiration, of unlabeled carbon at lower CO_2 concentrations and during flushing in CO_2-free air. This source could be previously fixed material and about 20% of the substrate would be supplied via (K) at 150 μl/l CO_2 and 40% at 53 μl/l CO_2 (Fig. 2). During evolution in CO_2-free air, 60% of the substrate would be supplied either directly via (J) or indirectly via (E) (F) and 40% via (K) to yield a relative specific radioactivity of about 60%. One problem with this proposal, which is difficult to envisage, is that it requires the simultaneous generation of substrate for CO_2 evolution from two sources with some means of regulating the two pathways. It is also difficult to explain why the relative specific radioactivity of $^{14}CO_2$ evolved into CO_2-free air appears to saturate at 60 to 80% even after 4 hr (8) or 6 hr (18) of $^{14}CO_2$ fixation.

If the fixation of $^{14}CO_2$ is performed in low oxygen (1.5%) the compounds of P_S and recent storage products become equal in specific radioactivity to the $^{14}CO_2$ supplied. Little, if any, carbon is diverted via (F) to R_L. When oxygen (21%) is again supplied carbon flow to R_L immediately occurs and the patterns of $^{14}CO_2$ evolution into CO_2-free air that are observed (Fig. 8) are similar to those observed after fixation of $^{14}CO_2$ in air.

When the leaf is darkened, light respiration (R_L) continues for some time after photosynthesis (P_S) has stopped. The result is the postillumination outburst of CO_2 (Figs. 3–5) which

has been interpreted as a remnant of the process(es) of CO_2 production that were occurring in the light (9, 13, 15, 30, 35). The fact that the relative specific radioactivity of this outburst was identical to the relative specific radioactivity of the $^{14}CO_2$ that was being evolved in the immediately preceding light period (Fig. 3, 4, 5) adds considerable support to this view. This outburst is probably derived largely from material in R_L and is not observed in 1% O_2 because (F) and (G) are inhibited (Fig. 9). After this outburst, the specific radioactivity of the $^{14}CO_2$ evolved in the dark declined to a steady value which was much less than the specific radioactivity of the $^{14}CO_2$ evolved in the light (Figs. 3 to 5) a finding in agreement with earlier results of Goldsworthy (18). Thus, the immediate substrates for dark respiration are quite different than those for light respiration.

The fact that the dark respiratory substrates become labeled with ^{14}C does not necessarily mean that ^{14}C is rapidly transferred to these compounds during fixation of $^{14}CO_2$ in the light. Equally possible is a rapid flow of ^{14}C into these compounds from recent storage products or P_S via (B) and (M) after the leaf is darkened (1, 4, 19, 31, 33). Even then, the transfer could not be large as these ^{14}C-labeled substrates were exhausted in about 25 min (Figs. 3–5), and then the specific radioactivity of the $^{14}CO_2$ evolved in the dark decreased. Eventually, the specific radioactivity of the $^{14}CO_2$ evolved in the light and dark becomes equal (Figs. 3 and 4) and at this time both substrates are presumably derived from previously fixed or remote storage products (Fig. 9).

When the leaf was flushed with normal air (300 μl/l CO_2) in the light $^{14}CO_2$ evolution rapidly ceased (Figs. 3–5). This does not mean that CO_2 evolution into CO_2-free air is greater than CO_2 evolution in normal air but only that the ^{14}C-labeled compounds in P_S and R_L (Fig. 9) are rapidly turned over and replaced with unlabeled carbon.

The evolution of $^{14}CO_2$ from a leaf, after a period of photosynthesis in $^{14}CO_2$, can provide much useful information on the relationship of the substrate for CO_2 evolution to the recent products of photosynthesis. It cannot, however, without a measurement of the specific radioactivity, provide, as Zelitch (38, 39) proposes, an estimate of either photorespiration or dark respiration. The specific radioactivity of $^{14}CO_2$ evolved in the light is usually much greater than the specific radioactivity of $^{14}CO_2$ evolved in the dark. Further, the relationship between the specific radioactivity of $^{14}CO_2$ evolved in the light and that evolved in the dark is not constant, as both depend on the total amount of $^{14}CO_2$ fixed and the time of flushing in CO_2-free air. Compared to CO_2 evolution into a CO_2-free air stream, we must conclude that $^{14}CO_2$ evolution is an inferior and misleading method for the assay of photorespiration or dark respiration since it suffers from the same limitations and criticisms and, in addition, is confounded by possible specific radioactivity changes.

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